

any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

***Amendments***

Please amend the application as follows:

***In the Claims:***

Please cancel claim 1, without prejudice to or disclaimer of the subject matter contained therein.

Please enter the following new claims 35-150:

35. (New) A method of producing a nucleic acid molecule comprising:

- (a) providing a first nucleic acid molecule comprising a first portion of a gene and at least a first recombination site;
- (b) providing a second nucleic acid molecule comprising a second portion of said gene and at least a second recombination site; and
- (c) forming a mixture between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second portions of said gene are operably linked to form a functional gene.

36. (New) The method of claim 35, wherein said gene encodes a selectable marker.

SUB D2  
cont

37. (New) The method of claim 35, wherein said gene is an antibiotic resistance gene.

38. (New) The method of claim 37, wherein said antibiotic resistance gene is selected from the group consisting of a chloramphenicol resistance gene, an ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

B' 39. (New) The method of claim 37, wherein said antibiotic resistance gene is a chloramphenicol resistance gene.

40. (New) The method of claim 35, wherein said first or second portion of said gene comprises a promoter.

SUB D3

41. (New) The method of claim 35, wherein said first and second portions of said gene are fragments of a structural gene.

42. (New) The method of claim 35, wherein said gene encodes a heterodimeric gene product.

43. (New) The method of claim 35, wherein said first and second recombination sites are selected from the group consisting of *lox* sites, *att* sites, and mutants thereof.

44. (New) The method of claim 35, wherein said first and second recombination sites are selected from the group consisting of *lox* sites and *att* sites.

45. (New) The method of claim 35, wherein said first and second recombination sites are *lox* sites.

46. (New) The method of claim 45, wherein said *lox* sites are *loxP* sites.

47. (New) The method of claim 35, wherein said first and second recombination sites are *att* sites.

48. (New) The method of claim 47, wherein said *att* sites are selected from the group consisting of *attB* sites, *attP* sites, *attL* sites and *attR* sites.

49. (New) The method of claim 35, wherein said first nucleic acid molecule or said second nucleic acid molecule further comprises at least one additional recombination site.

50. (New) The method of claim 49, wherein said at least one additional recombination site is selected from the group consisting of *lox* sites and *att* sites.

51. (New) The method of claim 49, wherein said at least one additional recombination site is at least one *lox* site or a mutant thereof.

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52. (New) The method of claim 49, wherein said at least one additional recombination site is a *lox* site.

53. (New) The method of claim 52, wherein said *lox* site is a *loxP* site.

54. (New) The method of claim 49, wherein said at least one additional recombination site is at least one *att* site or a mutant thereof.

55. (New) The method of claim 49, wherein said at least one additional recombination site is an *att* site.

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56. (New) The method of claim 55, wherein said *att* site is selected from the group consisting of an *attB* site, an *attP* site, an *attL* site and an *attR* site.

57. (New) The method of claim 35, wherein said first portion of said gene is located adjacent to said first recombination site.

SUB D4


58. (New) The method of claim 35, wherein said second portion of said gene is located adjacent to said second recombination site.

59. (New) The method of claim 35, wherein said first nucleic acid molecule or said second nucleic acid molecule comprises at least one cloning site.

60. (New) The method of claim 35, wherein said at least one recombination protein is selected from the group consisting of Cre, Int, IHF, Xis, FLP,  $\gamma\delta$ , Tn3 resolvase, Hin, Gin, Cin and combinations thereof.

61. (New) The method of claim 35, wherein said at least one recombination protein is Cre.

62. (New) The method of claim 35, wherein said at least one recombination protein is selected from the group consisting of Int, IHF and Xis.

 63. (New) The method of claim 35, wherein said at least one recombination protein is Int.

64. (New) The method of claim 35, wherein said at least one recombination protein is IHF.

65. (New) The method of claim 35, wherein said at least one recombination protein is Xis.

66. (New) The method of claim 35, wherein said first nucleic acid molecule or said second nucleic acid molecule or said third nucleic acid molecule is a vector.

67. (New) The method of claim 66, wherein said vector is an expression vector.

68. (New) The method of claim 35, wherein said first nucleic acid molecule or said second nucleic acid molecule is linear.

SUB D5

69. (New) The method of claim 35, wherein said first or said second portions of said gene are PCR products.

70. (New) The method of claim 35, further comprising expressing said functional gene.

71. (New) The method of claim 35, further comprising contacting at least one host cell with said mixture, and selecting for a host cell comprising said third nucleic acid molecule.

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72. (New) The method of claim 71, further comprising selecting against a host cell comprising said first or said second nucleic acid molecule.

73. (New) The method of claim 71, further comprising selecting against a host cell comprising said first and said second nucleic acid molecules.


74. (New) The method of claim 71, further comprising expressing said functional gene in said selected host cell.

75. (New) The method of claim 71, wherein said host cell is a prokaryotic cell.

76. (New) The method of claim 71, wherein said host cell is a bacterial cell.

77. (New) The method of claim 71, wherein said host cell is an *Escherichia coli* cell.

78. (New) A method of producing a nucleic acid molecule comprising:

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- (a) providing a first nucleic acid molecule comprising a first portion of an antibiotic resistance gene and at least a first recombination site;
  - (b) providing a second nucleic acid molecule comprising a second portion of said antibiotic resistance gene and at least a second recombination site; and
  - (c) forming a mixture between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second portions of said gene are operably linked to form a functional antibiotic resistance gene.

79. (New) The method of claim 78, wherein said antibiotic resistance gene is selected from the group consisting of a chloramphenicol resistance gene, an ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

80. (New) The method of claim 78, wherein said antibiotic resistance gene is a chloramphenicol resistance gene.

81. (New) The method of claim 78, wherein said first or second portion of said gene comprises a promoter.

82. (New) The method of claim 78, wherein said first and second recombination sites are selected from the group consisting of *lox* sites, *att* sites, and mutants thereof.

83. (New) The method of claim 78, wherein said first and second recombination sites are selected from the group consisting of *lox* sites and *att* sites.

84. (New) The method of claim 78, wherein said first and second recombination sites are *lox* sites.

85. (New) The method of claim 84, wherein said *lox* sites are *loxP* sites.

86. (New) The method of claim 78, wherein said first and second recombination sites are *att* sites.

87. (New) The method of claim 86, wherein said *att* sites are selected from the group consisting of *attB* sites, *attP* sites, *attL* sites and *attR* sites.

88. (New) The method of claim 78, wherein said first nucleic acid molecule or said second nucleic acid molecule further comprises at least one additional recombination site.

89. (New) The method of claim 88, wherein said at least one additional recombination site is selected from the group consisting of *lox* sites and *att* sites.



90. (New) The method of claim 88, wherein said at least one additional recombination site is at least one *lox* site or a mutant thereof.

91. (New) The method of claim 88, wherein said at least one additional recombination site is a *lox* site.

92. (New) The method of claim 91, wherein said *lox* site is a *loxP* site.

93. (New) The method of claim 88, wherein said at least one additional recombination site is at least one *att* site or a mutant thereof.

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94. (New) The method of claim 88, wherein said at least one additional recombination site is an *att* site.

95. (New) The method of claim 94, wherein said *att* site is selected from the group consisting of an *attB* site, an *attP* site, an *attL* site and an *attR* site.

96. (New) The method of claim 78, wherein said first portion of said gene is located adjacent to said first recombination site.

97. (New) The method of claim 78, wherein said second portion of said gene is located adjacent to said second recombination site.

98. (New) The method of claim 78, wherein said first nucleic acid molecule or said second nucleic acid molecule comprises at least one cloning site.

99. (New) The method of claim 78, wherein said at least one recombination protein is selected from the group consisting of Cre, Int, IHF, Xis, FLP,  $\gamma\delta$ , Tn3 resolvase, Hin, Gin, Cin and combinations thereof.

100. (New) The method of claim 78, wherein said at least one recombination protein is Cre.

101. (New) The method of claim 78, wherein said at least one recombination protein is selected from the group consisting of Int, IHF and Xis.

102. (New) The method of claim 78, wherein said first nucleic acid molecule or said second nucleic acid molecule or said third nucleic acid molecule is a vector.

103. (New) The method of claim 102, wherein said vector is an expression vector.

104. (New) The method of claim 78, wherein said first nucleic acid molecule or said second nucleic acid molecule is linear.

105. (New) The method of claim 78, wherein said first or said second portions of said gene are PCR products.

106. (New) The method of claim 78, further comprising contacting at least one host cell with said mixture, and selecting for a host cell comprising said third nucleic acid molecule.

107. (New) The method of claim 106, further comprising selecting against a host cell comprising said first or said second nucleic acid molecule.

108. (New) The method of claim 106, further comprising selecting against a host cell comprising said first and said second nucleic acid molecule.

109. (New) The method of claim 106, wherein said host cell is a prokaryotic cell.

110. (New) The method of claim 106, wherein said host cell is a bacterial cell.

111. (New) The method of claim 106, wherein said host cell is an *Escherichia coli* cell.

112. (New) The method of claim 78, further comprising introducing said third nucleic acid molecule into a host cell.

113. (New) The method of claim 78, further comprising introducing said third nucleic acid molecule into a host cell and expressing said antibiotic resistance gene.

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114. (New) The method of claim 113, wherein said host cell is an *Escherichia coli* cell.

115. (New) A method of producing a nucleic acid molecule comprising:

- SUB DG
- (a) providing a first nucleic acid molecule comprising at least one promoter and at least a first recombination site;
- (b) providing a second nucleic acid molecule comprising at least one antibiotic resistance gene and at least a second recombination site; and
- (c) forming a mixture between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said promoter and said antibiotic resistance gene are operably linked.
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116. (New) The method of claim 115, wherein said antibiotic resistance gene is selected from the group consisting of a chloramphenicol resistance gene, an ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

117. (New) The method of claim 115, wherein said antibiotic resistance gene is a chloramphenicol resistance gene.

118. (New) The method of claim 115, wherein said first and second recombination sites are selected from the group consisting of *lox* sites, *att* sites, and mutants thereof.

119. (New) The method of claim 115, wherein said first and second recombination sites are selected from the group consisting of *lox* sites and *att* sites.

120. (New) The method of claim 115, wherein said first and second recombination sites are *lox* sites.

121. (New) The method of claim 120, wherein said *lox* sites are *loxP* sites.

122. (New) The method of claim 115, wherein said first and second recombination sites are *att* sites.

123. (New) The method of claim 122, wherein said *att* sites are selected from the group consisting of *attB* sites, *attP* sites, *attL* sites and *attR* sites.

124. (New) The method of claim 115, wherein said first nucleic acid molecule or said second nucleic acid molecule further comprises at least one additional recombination site.

125. (New) The method of claim 124, wherein said at least one additional recombination site is selected from the group consisting of *lox* sites and *att* sites.

126. (New) The method of claim 124, wherein said at least one additional recombination site is at least one *lox* site or a mutant thereof.

127. (New) The method of claim 124, wherein said at least one additional recombination site is a *lox* site.

128. (New) The method of claim 127, wherein said *lox* site is a *loxP* site.

129. (New) The method of claim 124, wherein said at least one additional recombination site is at least one *att* site or a mutant thereof.

130. (New) The method of claim 124, wherein said at least one additional recombination site is an *att* site.

131. (New) The method of claim 130, wherein said *att* site is selected from the group consisting of an *attB* site, an *attP* site, an *attL* site and an *attR* site.

132. (New) The method of claim 115, wherein said promoter is located adjacent to said first recombination site.

133. (New) The method of claim 115, wherein said antibiotic resistance gene is located adjacent to said second recombination site.

134. (New) The method of claim 115, wherein said first nucleic acid molecule or said second nucleic acid molecule comprises at least one cloning site.

135. (New) The method of claim 115, wherein said at least one recombination protein is selected from the group consisting of Cre, Int, IHF, Xis, FLP,  $\gamma\delta$ , Tn3 resolvase, Hin, Gin, Cin and combinations thereof.

136. (New) The method of claim 115, wherein said at least one recombination protein is Cre.

137. (New) The method of claim 115, wherein said at least one recombination protein is selected from the group consisting of Int, IHF and Xis.

138. (New) The method of claim 115, wherein said first nucleic acid molecule or said second nucleic acid molecule or said third nucleic acid molecule is a vector.

139. (New) The method of claim 138, wherein said vector is an expression vector.

140. (New) The method of claim 115, wherein said first nucleic acid molecule or said second nucleic acid molecule is linear.

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141. (New) The method of claim 115, wherein said first or said second portions of said gene are PCR products.

142. (New) The method of claim 115, further comprising contacting at least one host cell with said mixture, and selecting for a host cell comprising said third nucleic acid molecule.

143. (New) The method of claim 142, further comprising selecting against a host cell comprising said first or said second nucleic acid molecule.

144. (New) The method of claim 142, further comprising selecting against a host cell comprising said first and said second nucleic acid molecule.

145. (New) The method of claim 142, wherein said host cell is a prokaryotic cell.

146. (New) The method of claim 142, wherein said host cell is a bacterial cell.

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C. coli

147. (New) The method of claim 142, wherein said host cell is an *Escherichia coli* cell.

148. (New) The method of claim 115, further comprising introducing said third nucleic acid molecule into a host cell.

SUB D9

149. (New) The method of claim 115, further comprising introducing said nucleic acid molecule into a host cell and expressing said antibiotic resistance gene.

150. (New) The method of claim 149, wherein said host cell is an *Escherichia coli* cell.

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